IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Timothy Tak Chun YIP *et al.*

Title: **SERUM BIOMARKERS IN LUNG CANCER**

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DECLARATION OF LEE LOMAS UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450
Sir:

I, Lee Lomas, state and declare that:

1. I am the separations R&D manager, laboratory separations division of Bio-Rad Laboratories Inc. I have no personal interest in the disposition of the captioned application.

2. I received my Ph.D in 1994 from the University of Alberta, Canada, in Biological Sciences. In 1998 I completed my postdoctoral work at the Department of Biochemistry, University of Liverpool (U.K.), with a focus on peptide regulation of hormonal development in blood-feeding arthropods (ticks).

3. In 1998 I joined Ciphergen, Inc. as an applications development scientist. (Ciphergen later changed its name to Vermillion, Inc., which is the assignee of the subject application.) My position at Ciphergen involved my working directly with customers to develop biomarker discovery and protein purification protocols, tailored to a customer's individual needs. After 2001 I had transitioned to the company's R&D office in the U.S., initially as manager of the applications group and then as overall chemistry R&D manager. In the latter role I oversaw reagent development, including protein chip arrays,

resin columns, and buffer consumables. In 2008 I joined Bio-Rad Laboratories as R&D manager of laboratory separations, overseeing a group tasked with developing reagents and kits for the characterization, separation/purification, and identification of proteins to the life science and pharma markets.

4. Based on the experience summarized above, I am aware that the use of two or more approximating techniques is commonplace in the context of rapid screening for the presence of an analyte; this, in order to obtain complementary information that, taken together, provides sufficient confidence in its presumed identity. This approach is particularly apropos when, as is often the case, methodology for confirming analyte identity unambiguously is either unavailable or too laborious for routine implementation. In recombinant protein production, for example, one or more modes of chromatography, such as size exclusion, anionic exchange, cationic exchange, hydrophobic or hydrophilic binding, metal affinity, or epitope affinity, often are combined with mass determination (*e.g.*, mass spectrometry or polyacrylamide gel electrophoresis) in order to detect a putative target protein in a complex starting sample.

5. By virtue of the experience summarized above, I also am aware that immobilized metal affinity chromatography (IMAC) and weak cationic exchange (WCX) chromatography are techniques commonly used to isolate populations of proteins on the basis of a biophysical/chemical property. IMAC relies on the affinity of a target protein to a metal molecule (*e.g.*, Fe^{3+}, Cu^{2+}, Ga^{3+}), coordinated by iminodiacetic acid or nitrilotriacetic acid ligand. WCX chromatography relies on the charge state of a protein, determined by its pI value, in a binding buffer of defined pH. Although there exist multiple manufacturers of both IMAC and WCX supports, the fundamental principles of operation are the same for all, and methodologies developed for one format (*e.g.*, an IMAC array charged with Cu^{2+}) readily can be transferred to another (*e.g.*, resin/beads charged with Cu^{2+}). Conversely, in my experience there is no widespread understanding or concern in the field that a manufacturer may change the labeling for or the characteristics of an IMAC- or WCX-based assay in the future, as different formats are created.

6. I am aware as well that, during a biomarker discovery phase, candidate markers are enriched and detected from a complex sample, using a physical/chemical "signature" that

is based on their chromatographic binding characteristic and their unique mass. Although other methods eventually may be employed to fix the identity of these markers unambiguously, a combination of their physical/chemical signatures provides sufficient information to infer identity in routine analysis. Indeed, tools such as TagIdent (http://expasy.org/tools/tagident.html) are used routinely to help identify proteins based on physical/chemical signatures such as molecular weight and isoelectric point (pI), which can be deduced from a combination of mass spectrometry and cation exchange properties. For example, by altering the pH of the elution buffer used with a chromatographic resin, either a SELDI array or bead, the pI of a biomarker can be deduced. By definition, its mass is provided by the m/z value of the peak obtained by mass spectrometry. Such routine analysis across hundreds to thousands of samples, utilizing a combination of IMAC, WCX and SELDI signatures, for example, is deemed sufficiently precise to allow for the validation of a biomarker, even in the absence of a definitive identity.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date May 17, 2010 By Lee Lomas
Lee Lomas